Journal of Infection 88 (2024) 106162

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Bacteria and Bacterial Diseases

Risk factors for *Nocardia* infection among allogeneic hematopoietic cell transplant recipients: A case-control study of the Infectious Diseases Working Party of the European Society for Blood and Marrow Transplantation

Julien De Greef [a,1], Dina Averbuch [b,1], Laura Tondeur [c,2], Amélie Duréault [d,2], Tsila Zuckerman [e,f], Xavier Roussel [g], Christine Robin [h], Alienor Xhaard [i], Simona Pagliuca [j], Yves Beguin [k], Carmen Botella-Garcia [l], Nina Khanna [m], Amandine Le Bourgeois [n], Jens Van Praet [o], Aloysius Ho [p], Nicolaus Kröger [q], Sophie Ducastelle Leprêtre [r], Damien Roos-Weil [s], Mahmoud Aljurf [t], Nicole Blijlevens [u], Igor Wolfgang Blau [v], Kristina Carlson [w], Matthew Collin [x], Arnold Ganser [y], Alban Villate [z], Johannes Lakner [aa], Sonja Martin [ab], Arnon Nagler [ac], Ron Ram [ad], Anna Torrent [ae], Maria Stamouli [af], Malgorzata Mikulska [ag,ah], Lidia Gil [ai], Lotus Wendel [ai], Gloria Tridello [ai], Nina Knelange [ai], Rafael de la Camara [aj,ak], Olivier Lortholary [d], Arnaud Fontanet [c,al], Jan Styczynski [am], Johan Maertens [an], Julien Coussement [ao,ap,*,3], David Lebeaux [aq,ar,3]

[a] *Department of Internal Medicine and Infectious Diseases, Cliniques universitaires Saint-Luc, Université catholique de Louvain (UCLouvain), Brussels, Belgium*
[b] *Pediatric Infectious Diseases, Faculty of Medicine, Hebrew University of Jerusalem, Hadassah Medical Center, Jerusalem, Israel*
[c] *Emerging Diseases Epidemiology Unit, Institut Pasteur, Université Paris Cité, 75015 Paris, France*
[d] *Centre d'Infectiologie Necker Pasteur, Hôpital Necker-Enfants Malades, Assistance Publique - Hôpitaux de Paris, Université Paris Descartes, Paris, France*
[e] *Rambam Health Care Campus, Haifa, Israel*
[f] *Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*
[g] *Department of Hematology, University of Franche-Comte, INSERM UMR1098, Besançon University Hospital, Besançon, France*
[h] *Department of Hematology, Henri Mondor University Hospital, Creteil, France*
[i] *Hematology-Transplantation, Hospital St-Louis, Paris Diderot University, Paris, France*
[j] *Hematology Department, Nancy University Hospital, Vandoeuvre-lès-Nancy, France*
[k] *Centre Hospitalier Universitaire of Liège and University of Liège, Liège, Belgium*
[l] *Centre Hospitalier Universitaire Bordeaux, Bordeaux, France*
[m] *Division of Infectious Diseases and Hospital Epidemiology, University and University Hospital of Basel, Basel, Switzerland*
[n] *Centre Hospitalier Universitaire Nantes, Nantes, France*
[o] *Department of Nephrology and Infectious Diseases, Algemeen Ziekenhuis Sint-Jan Brugge-Oostende, Brugge, Belgium*
[p] *Singapore General Hospital, Singapore*
[q] *Department of Stem Cell Transplantation, University Medical Center, Hamburg, Germany*
[r] *Hôpital Lyon Sud, Hospices Civils de Lyon, Pierre Bénite, France*
[s] *Sorbonne Université, Hôpital Pitié-Salpêtrière, Paris, France*
[t] *King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia*
[u] *Department of Hematology, Radboud University Medical Center, Nijmegen, the Netherlands*
[v] *Campus Virchow Klinikum CVK, Berlin, Germany*
[w] *Dept of Haematology, University Hospital, Uppsala, Sweden*
[x] *Nordern Centre for Bone Marrow Transplantation Freeman Hospital - Adult HSCT Unit, Newcastle, United Kingdom*
[y] *Department of Hematology Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany*
[z] *Service d'hématologie et thérapie cellulaire, Centre Hospitalier Universitaire de Tours, Université de Tours, Tours, France*
[aa] *Medical Clinic III, University Medical Center, Rostock, Germany*
[ab] *Robert-Bosch-Krankenhaus, Stuttgart, Germany*
[ac] *Chaim Sheba Medical Center, Tel Hashomer, Israel*
[ad] *Tel Aviv Sourasky Medical Center and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*
[ae] *ICO-Hospital Germans Trias i Pujol, Josep Carreras Research Institute, Badalona, Spain*
[af] *Attikon University General Hospital, Athens, Greece*
[ag] *Division of Infectious Diseases, Department of Health Sciences, University of Genoa, Genoa, Italy*

* Corresponding author at: Service de Maladies Infectieuses et Tropicales, Centre Hospitalier universitaire de Guadeloupe, Les Abymes, Guadeloupe, France.
E-mail address: julien.coussement@chu-guadeloupe.fr (J. Coussement).

https://doi.org/10.1016/j.jinf.2024.106162

[ah] IRCCS Ospedale Policlinico San Martino, Genoa, Italy
[ai] European Society for Blood and Marrow Transplantation (EBMT), Leiden Study Unit, Leiden, the Netherlands
[aj] Hospital de la Princesa, Madrid, Spain
[ak] Infectious Diseases Working Party, EBMT, Spain
[al] Unité PACRI, Conservatoire National des Arts et Métiers, 75003 Paris, France
[am] Department of Pediatric Hematology and Oncology, Collegium Medicum, Nicolaus Copernicus University Torun, Bydgoszcz, Poland
[an] Department of Hematology, Universitaire Ziekenhuizen Leuven, Leuven, Belgium
[ao] Sir Peter MacCallum Department of Oncology, The University of Melbourne, Victoria, Australia
[ap] Service de Maladies Infectieuses et Tropicales, Centre Hospitalier universitaire de Guadeloupe, Les Abymes, Guadeloupe, France
[aq] Institut Pasteur, Université Paris Cité, CNRS UMR 6047, Genetics of Biofilms Laboratory, 75015 Paris, France
[ar] Département de Maladies Infectieuses et Tropicales, AP-HP, Hôpital Saint-Louis, Lariboisière, F-75010 Paris, France

ARTICLE INFO

Article history:
Accepted 15 April 2024
Available online 23 April 2024

Keywords:
Nocardiosis
Hematopoietic stem cell transplantation
HSCT

SUMMARY

Objectives: Nocardiosis is a rare but life-threatening infection after hematopoietic cell transplantation (HCT). We aimed at identifying risk factors for nocardiosis after allogeneic HCT and clarifying the effect of trimethoprim-sulfamethoxazole prophylaxis on its occurrence.
Methods: We performed a retrospective multicenter case-control study of patients diagnosed with nocardiosis after allogeneic HCT between January 2000 and December 2018. For each case, two controls were matched by center, transplant date, and age group. Multivariable analysis was conducted using conditional logistic regression to identify potential risk factors for nocardiosis. Kaplan-Meier survival curves of cases and controls were compared using log-rank tests.
Results: Sixty-four cases and 128 controls were included. Nocardiosis occurred at a median of 9 months after allogeneic HCT (interquartile range: 5–18). After adjustment for potential confounders in a multivariable model, *Nocardia* infection was associated with tacrolimus use (adjusted odds ratio [aOR] 9.9, 95 % confidence interval [95 % CI]: 1.6–62.7), lymphocyte count < 500/µL (aOR 8.9, 95 % CI: 2.3–34.7), male sex (aOR 8.1, 95 % CI: 2.1–31.5), recent use of systemic corticosteroids (aOR 7.9, 95 % CI: 2.2–28.2), and recent CMV infection (aOR 4.3, 95 % CI: 1.2–15.9). Conversely, use of trimethoprim-sulfamethoxazole prophylaxis was associated with a significantly decreased risk of nocardiosis (aOR 0.2, 95 % CI: 0.1–0.8). HCT recipients who developed nocardiosis had a significantly decreased survival, as compared with controls (12-month survival: 58 % and 90 %, respectively; p < 0.0001).
Conclusions: We identified six factors independently associated with the occurrence of nocardiosis among allogeneic HCT recipients. In particular, trimethoprim-sulfamethoxazole prophylaxis was found to protect against nocardiosis.

Introduction

Nocardia is a ubiquitous environmental Gram-positive bacterium that may cause life-threatening infection in humans.[1] Transplant recipients and other compromised hosts are at increased risk of *Nocardia* infection.[2] In particular, the association between nocardiosis and allogeneic hematopoietic cell transplantation (HCT) has been documented since the late 1970s,[3,4] and retrospective studies found nocardiosis to occur after 0.3 to 1.7 % of allogeneic HCTs.[5,6]

While several case-control studies have investigated potential risk factors for nocardiosis in solid organ transplant (SOT) recipients,[7–9] only limited data are available for allogeneic HCT recipients. To our knowledge, only two limited size case-control studies have examined the potential effect of trimethoprim-sulfamethoxazole (TMP-SMX) in the prevention of nocardiosis after allogeneic HCT.[10,11] Limitations of these two studies include the low number of included patients (11 and 20 patients with nocardiosis, respectively), and the fact that one of them was an unpublished conference abstract. The most recent of these two studies found antimicrobial prophylaxis to significantly prevent nocardiosis after allogeneic HCT. Interestingly, although antimicrobial prophylaxis was defined as the use of either TMP-SMX or doxycycline for the purpose of the multivariable analysis, the authors reported that none (0 %) of the 20 nocardiosis cases were receiving TMP-SMX at diagnosis, as compared with 30 (37.5 %) of the 80 controls. Overall, published data are limited and whether TMP-SMX effectively prevents nocardiosis in HCT recipients remains an unanswered question. Taking advantage of a recently published retrospective multinational study describing the characteristics of HCT recipients with nocardiosis,[6] we performed a case-control study aiming at identifying potential risk factors for nocardiosis in allogeneic HCT recipients.

Methods

Study design and setting

This was an international retrospective study supported by the Infectious Diseases Working Party (IDWP) of the European Society for Blood and Marrow Transplantation (EBMT). All EBMT-affiliated centers were invited to participate (more details provided in the parent study[6]). For case identification, participating centers were requested to systematically screen local microbiology and HCT databases. Study data were obtained either using the EBMT database or through a dedicated case report form when not included in the

[1] JDG and DA contributed equally to this work (co-first authors).
[2] LT and AD contributed equally to this work (co-second authors).
[3] JC and DL contributed equally to this work (co-senior authors).

database. This study was performed in accordance with the appropriate regulations in the participating countries, including approval by the local ethical committees.

Inclusion criteria

Patients meeting all the following criteria were included as cases: (1) history of allogeneic HCT; (2) isolation of *Nocardia* spp. in a clinical sample, after HCT (i.e., from start of conditioning); (3) clinical and/or radiological signs compatible with nocardiosis; (4) diagnosis made between 1 January 2000 and 31 December 2018. Two matched controls were selected for each case. Matched controls were allogeneic HCT recipients who (1) underwent transplantation in the same center as the case; (2) had no evidence of *Nocardia* infection during the study follow-up; (3) had received their transplant as close as possible to the case's date of transplant; (4) had survived at least as long as the case had prior to the diagnosis of *Nocardia* infection; (5) belonged to the same age category as the case (< versus ≥ 18 years). Controls were independently and centrally selected by the IDWP office of EBMT.

Microbiology

Diagnosis of nocardiosis required growth of *Nocardia* in a clinical sample. For inclusion in the present study, identification of *Nocardia* at the genus level had to be confirmed using molecular methods (i.e. gene sequencing, see Supplementary Appendix) and/or Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight spectrometry (MALDI-TOF); other cases were excluded.[12,13] Further details on identification of *Nocardia* at the species level are provided as Supplementary Appendix.

Clinical data

The date of diagnosis of nocardiosis was defined as the day on which the first clinical sample (e.g., sputum) yielding *Nocardia* species was collected. For control patients, a corresponding date was chosen on the basis of their matched case's date of diagnosis, to obtain a similar time from transplantation. We collected clinical data with a specific focus on possible risk factors for nocardiosis, including data on the underlying hematological disease(s), comorbidities which were known at time of transplantation and time of nocardiosis, HCT (including conditioning regimen, donor characteristics, stem cell origin, and graft function), and possible post-transplant complications including infections and acute graft versus host disease (aGVHD) or chronic GVHD (cGVHD). We also collected detailed therapeutic data, including on antimicrobial prophylaxis, targeted immunotherapeutic agents and immunomodulatory drugs (see Table 1 for time frames used and other details on definitions used). We further collected biological data at time of nocardiosis, such as lymphocyte and neutrophil counts, and data on the possible history of documented bacterial, viral and invasive fungal infections within six months before nocardiosis. Lastly, we recorded outcome data (date of last follow-up or death) and determined 12-month all-cause mortality after diagnosis of *Nocardia* infection. Whether death was attributable to nocardiosis was assessed by local investigators.

Statistical analysis

Continuous data are presented as medians with their interquartile ranges (IQR) and categorical data as counts and proportions. Continuous variables were compared across groups using Mann-Whitney U tests. Proportions were compared using chi-squared or Fischer's Exact tests. Independent risk factors for nocardiosis were identified by multivariable conditional logistic regression to account for the matching strategy. All variables of the univariable analysis were included in the multivariable model. Eventually, only variables with p values < 0.05 were retained in the final multivariable model. TMP-SMX prophylaxis was included in the multivariable analysis independently of the p-value, due to its relevance and the ongoing debate on its protective effect. Multiple imputations were performed to replace missing values using the multivariable imputation by chained equations method. In addition, a complete-case sensitivity analysis was undertaken, using subjects with no missing data for any relevant variable. Kaplan-Meier curves and log-rank tests were used to compare 12-month survival between cases and controls. All statistical analyses were performed using Stata® 17.0 (StataCorp, College Station, TX, USA).

Results

Characteristics of cases and controls

We included 64 HCT recipients with nocardiosis, and 128 matched controls. Patient characteristics are shown in Table 1. Nocardiosis occurred at a median of 9.3 months (IQR: 4.8–18.1) after HCT. Only two cases and their four relative controls were younger than 18 years. Overall, most patients belonged to a low-risk group at the time of HCT according to Hematopoietic Cell Transplantation Comorbidity Index[14] and received peripheral blood stem cells. Active aGVHD was present in 17.2 % and active cGVHD in 39.1 % of cases. Characteristics of cases are detailed in Supplementary Table 1 and Supplementary Fig. 1**.** Among 50 tested *Nocardia* isolates, 92 % were found to be susceptible to TMP-SMX (Supplementary Table 2). Details on the countries of origin, matching characteristics and description of GVHD grades can be found in the Supplementary Appendix.

Risk factors for nocardiosis

In univariate analysis (Table 2), male sex, presence of moderate or severe pulmonary disease, underlying hematological diseases, active aGVHD (any grade), active cGVHD (any grade), recent invasive fungal infection, recent bacterial infection, recent CMV infection, receipt of systemic corticosteroids within previous 12 months, ongoing tacrolimus treatment and lymphocyte count < 500 cells/mm^3 were significantly associated with the development of nocardiosis.

On multivariable analysis (Table 2), we found ongoing tacrolimus use (P = 0.02), lymphocyte count < 500/mm^3 (P = 0.002), male sex (P = 0.003), treatment with systemic corticosteroids within previous 12 months (P = 0.001) and CMV infection in the previous 6 months (P = 0.03) to be independently associated with the occurrence of nocardiosis after HCT. Conversely, ongoing use of TMP-SMX prophylaxis (P = 0.02) was significantly associated with a decreased risk of nocardiosis after HCT. The protective effect of TMP-SMX was masked in univariable analysis by the higher proportion of patients taking steroids among those taking TMP-SMX versus those not taking TMP-SMX (66.7 % versus 45.5 %, respectively; P = 0.004), steroids being an important risk factor for nocardiosis (OR 8.89, 95 % CI: 3.7–21.3) (negative confounding effect). After adjustment for steroids intake, the OR of TMP-SMX prophylaxis decreased from 0.76 (95 % CI: 0.37–1.54) in univariable analysis to 0.36 (95 % CI: 0.15–0.87) in bivariable analysis. Median weekly TMP-SMX dose, expressed in mg of TMP, was 480 mg (IQR 440–520) for cases and 480 mg (IQR 480–500) for controls (P = 0.69) (Supplementary Fig. 2).

These findings were confirmed in our complete-case sensitivity analysis (Supplementary Table 3).

Survival analysis

Survival status one year after nocardiosis diagnosis was available for 97 % of cases [62/64] and 95 % of controls (121/128). One-year all-

Table 1
Clinical and biological characteristics of 64 allogeneic hematopoietic cell transplant (HCT) recipients with nocardiosis (cases) and 128 control HCT recipients who did not develop nocardiosis during the study follow-up.[a]

	HCT recipient with nocardiosis (cases) N = 64 *n (%)*	HCT recipient without nocardiosis (controls) N = 128 *n (%)*	Total N = 192 *n (%)*	P-value
Demographics				
Age, *median (IQR) (years)*	52.6 (42.8–58.9)	50.0 (37.4–59.6)	50.9 (38.8–59.5)	0.51
Male	49 (76.6)	67 (52.3)	116 (60.4)	0.001
Active comorbidities[b]				
Moderate/severe renal disease	4 (6.3)	1 (0.8)	5 (2.6)	0.04
Moderate/severe pulmonary disease	12 (18.7)	11 (8.6)	23 (12.0)	0.04
HCT-CI score[c]				
Low risk (Score = 0)	47 (73.4)	102 (79.6)	149 (77.6)	0.61
Intermediate risk (Score = 1-2)	8 (12.5)	13 (10.2)	21 (10.9)	
High risk (Score ≥ 3)	9 (14.1)	13 (10.2)	22 (11.5)	
Underlying diseases				
ALL and related leukemias[d]	4 (6.3)	15 (11.7)	19 (9.9)	< 0.001
AML	12 (18.7)	50 (39.1)	62 (32.3)	
Lymphoid malignancies	30 (46.9)	28 (21.9)	58 (30.2)	
MDS/MPN/CML	18 (28.1)	28 (21.9)	46 (24.0)	
Others	0 (0)	7 (5.4)	7 (3.6)	
HCT Characteristics				
Stem cell source				
Bone marrow	12 (18.7)	18 (14.1)	30 (15.6)	0.73
Peripheral blood	51 (79.7)	108 (84.4)	159 (82.8)	
Cord blood	1 (1.6)	2 (1.5)	3 (1.6)	
Donor type				
Identical sibling or MRD	26 (40.6)	62 (48.4)	88 (45.8)	0.35
Matched unrelated donor	21 (32.8)	28 (21.9)	49 (25.6)	
MMRD or MMUD	8 (12.5)	22 (17.2)	30 (15.6)	
Unrelated, number of mismatches unknown	9 (14.1)	16 (12.5)	25 (13.0)	
Conditioning regimen *(n = 189)*				
Myeloablative	31 (48.4)	56 (43.8)	87 (45.3)	0.63
Non-myeloablative	33 (51.6)	69 (53.9)	102 (53.1)	
TBI				
No TBI or TBI < 8 Gy	51 (79.7)	102 (79.7)	153 (79.7)	0.99
TBI ≥ 8 Gy	13 (20.3)	26 (20.3)	39 (20.3)	
T cell depletion *(n = 185)*				
No T cell depletion	22 (34.4)	48 (37.5)	70 (36.5)	0.43
In-vivo T cell depletion	39 (60.9)	63 (49.2)	102 (53.1)	
Ex-vivo T cell depletion	3 (4.7)	10 (7.8)	13 (6.8)	
Disease status at the time of *Nocardia*				
Other responses than complete remission	14 (21.9)	19 (14.8)	33 (17.2)	0.22
Complete Remission	50 (78.1)	109 (85.2)	159 (82.8)	
Other complications				
Occurrence of aGVHD[e] *(n = 178)*				
Active aGVHD	11 (17.2)	8 (6.3)	19 (9.9)	0.02
Occurrence of aGVHD during last 6 months	17 (26.6)	16 (12.5)	33 (17.2)	0.01
Active cGVHD[e] *(n = 184)*	25 (39.1)	19 (14.8)	44 (22.9)	< 0.001
Recent invasive fungal infection[f]	16 (25.0)	6 (4.7)	22 (11.5)	< 0.001
Recent bacterial infection[f]	25 (39.1)	29 (22.7)	54 (28.1)	0.02
Recent CMV infection[f]	24 (37.5)	33 (25.8)	57 (29.7)	0.08
Therapeutic characteristics *(n = 188)*				
Any systemic steroid treatment in the last 12 months	54 (84.4)	51 (39.8)	105 (54.7)	< 0.001
Ongoing systemic steroid treatment	45 (70.3)	35 (27.3)	80 (41.7)	< 0.001
Ongoing cyclosporine treatment	17 (26.6)	42 (32.8)	59 (30.7)	0.38
Ongoing tacrolimus treatment	21 (32.8)	7 (5.5)	28 (14.6)	< 0.001
Ongoing TMP-SMX prophylaxis	26 (40.6)	58 (45.3)	84 (43.7)	0.50
Biological Characteristics				
Lymphopenia < 500 /mm^3 *(n = 185)*	33 (51.6)	17 (13.3)	50 (26.0)	< 0.001
Neutropenia < 500 /mm^3 *(n = 185)*	1 (1.6)	2 (1.6)	3 (1.6)	0.99

NOTE: All variables are reported at the time of *Nocardia* infection for cases or matched time for controls, unless otherwise stated.
n: number of data analyzed (when < 192). Missing data categories were not included in P-values computation
Abbreviations: aGVHD: acute Graft versus Host Disease; ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; cGVHD: chronic aGVHD: acute Graft versus Host Disease; CML: Chronic myeloid leukemia; CMV: cytomegalovirus; HCT-CI: Hematopoietic Cell Transplantation-Comorbidity Index; HCT: hematopoietic stem cell transplantation; IQR: interquartile range; MDS: myelodysplastic syndrome; MMRD: mismatched unrelated donor; MMUD: mismatched unrelated donor; MPN: myeloproliferative neoplasm; MRD: Matched related donor; TBI: total body irradiation; TMP-SMX: trimethoprim-sulfamethoxazole.
[a] For control patients, a corresponding date was chosen on the basis of their matched case's date of diagnosis, to obtain a similar time from transplantation.
[b] Comorbidities are defined according to HCT-CI score[14].
[c] At the time of HCT.
[d] ALL and related leukemias: Acute lymphoblastic leukemia and mixed phenotype leukemia.
[e] Any grade of aGVHD or cGVHD.
[f] During the 6 months preceding the time of *Nocardia* infection for cases or matched time for controls.

cause mortality was significantly higher among cases than control HCT recipients who did not develop nocardiosis (44 % [27/62] versus 11 % [13/121, respectively); see Fig. 1 for survival curves (log-rank test $P < 0.0001$ for comparison of survival curves). A minority of deaths which occurred in cases were considered by the treating physician to be related to nocardiosis (30 % [8/27]).

Table 2
Risk factors for nocardiosis in 64 allogeneic hematopoietic cell transplant recipients cases compared to 128 controls who did not develop nocardiosis during the study follow-up,[a] after univariable and multivariable analysis by conditional logistic regression.

	Univariable OR (95 % CI)	P-value	Multivariable OR (95 % CI)	P-value
Clinical Characteristics				
Age category (years)				
≤ 35	Ref.	0.49		
]35-50]	1.9 (0.7–5.6)			
]50-65]	2.2 (0.8–5.9)			
> 65	1.9 (0.5–7.4)			
Sex				
Female	Ref.	0.001	Ref.	0.003
Male	3.0 (1.5–6.1)		8.1 (2.1–31.5)	
Active comorbidities[b]				
Moderate/severe renal disease	8.0 (0.9–71.6)	0.06		
Moderate/severe pulmonary disease	2.6 (1.0–6.5)	0.04		
HCT-CI score[c]				
Low risk (Score=0)	Ref.	0.53		
Intermediate risk (Score=1-2)	1.4 (0.5–3.8)			
High risk (Score ≥ 3)	1.7 (0.6–5.0)			
Underlying diseases				
ALL and related leukemias[d]	Ref.	0.0002		
AML	0.6 (0.2–2.3)			
Lymphoid malignancies	4.3 (1.2–14.8)			
MDS/MPN/CML	2.8 (0.7–10.3)			
HCT characteristics				
Stem cell source				
Bone marrow	Ref.	0.68		
Peripheral blood	0.7 (0.3–1.6)			
Cord blood	0.7 (0.1–8.9)			
Donor type				
Identical sibling or MRD	Ref.	0.27		
Matched unrelated donor	2.0 (0.9–4.5)			
MMRD or MMUD	0.8 (0.3–2.1)			
Unrelated, number of mismatches unknown	1.6 (0.4–7.0)			
Conditioning regimen				
Myeloablative	Ref.	0.64		
Non-myeloablative	0.9 (0.4–1.7)			
Total body irradiation				
No, or < 8 Gy	Ref.	0.99		
≥ 8 Gy	1.0 (0.5–2.2)			
T cell depletion				
No	Ref.	0.30		
In-vivo	1.6 (0.7–3.4)			
Ex-vivo	0.5 (0.1–3.0)			
Disease status at the time of *Nocardia* infection				
Response other than complete remission	Ref.	0.24		
Complete remission	0.6 (0.3–1.4)			
Post-transplant complications				
Active aGVHD[e]	3.6 (1.2–10.6)	0.02		
Active cGVHD[e]	4.6 (2.0–10.4)	0.0001		
Recent invasive fungal infection[f]	6.1 (2.2–16.8)	0.0001		
Recent bacterial infection[f]	2.6 (1.3–5.4)	0.01		
Recent CMV infection[f]	2.3 (1.0–5.0)	0.04	4.3 (1.2–15.9)	0.03
Treatments received				
Any systemic steroid treatment in the last 12 months	8.9 (3.7–21.3)	< 0.0001	7.9 (2.2–28.2)	0.001
Ongoing cyclosporine treatment	0.7 (0.3–1.5)	0.32		
Ongoing tacrolimus treatment	12.4 (3.7–41.8)	< 0.0001	9.9 (1.6–62.7)	0.02
Ongoing TMP-SMX prophylaxis	0.8 (0.4–1.5)	0.45	0.2 (0.1–0.8)	0.02
Biological characteristics				
Lymphopenia < 500 /mm^3	8.4 (3.5–20.5)	< 0.0001	8.9 (2.3–34.7)	0.002
Neutropenia < 500 /mm^3	1.0 (0.1–11.0)	0.99		

NOTE: All variables are reported at the time of *Nocardia* infection for cases or matched time for controls, unless otherwise stated. Collinearity among independent variables was assessed through correlation coefficients and variance inflation factors; no significant collinearity was detected for our final multivariable model.
Abbreviations: aGVHD: acute Graft versus Host Disease; AML: Acute myeloid leukemia; cGVHD: Chronic Graft versus Host Disease; CI: Confidence Interval; CML: Chronic myeloid leukemia; CMV: cytomegalovirus; HCT-CI: Hematopoietic Cell Transplantation-Comorbidity Index; HCT: hematopoietic stem cell transplantation; IQR: interquartile range; MDS: myelodysplastic syndrome; MMRD: mismatched unrelated donor; MMUD: mismatched unrelated donor; MPN: myeloproliferative neoplasm; MRD: Matched related donor; OR: Odds ratio; TMP-SMX: trimethoprim-sulfamethoxazole.

[a] For control patients, a corresponding date was chosen on the basis of their matched case's date of diagnosis, to obtain a similar time from transplantation.
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[f] During the 6 months preceding the time of *Nocardia* infection for cases or matched time for controls.

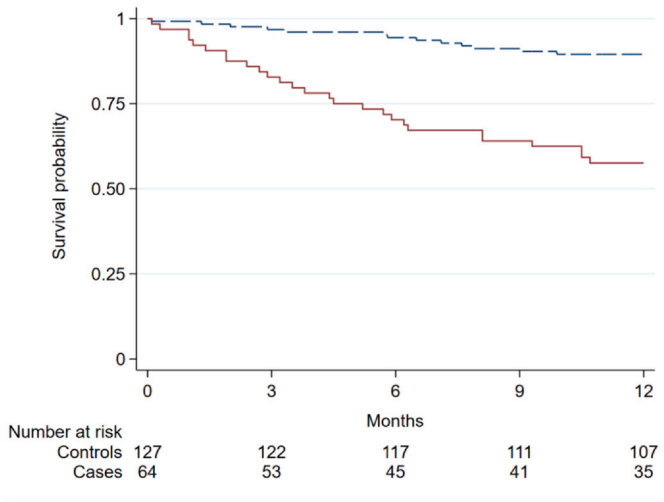

Fig. 1. One-year survival curves after diagnosis of nocardiosis occurring after allogeneic hematopoietic cell transplantation. Survival of nocardiosis (cases, n = 64, red solid line) and matched control transplant recipients (controls, n = 128, blue dotted line) was assessed using Kaplan-Meier curves and compared using log-rank tests.

Discussion

We report the results of a multinational case-control study of nocardiosis after allogeneic HCT, which to our knowledge is the largest study on this topic. Careful matching of HCT recipients who developed nocardiosis to control HCT recipients allowed us to identify several variables associated with the occurrence of this opportunistic infection in multivariable analysis: ongoing use of tacrolimus, lymphopenia, receipt of corticosteroids within previous 12 months, CMV infection within previous 6 months and male sex. In contrast, use of TMP-SMX prophylaxis was found to be associated with a reduced risk of post-transplant nocardiosis.

In line with previous case-control studies performed in SOT and allogeneic HCT recipients, we found factors related to immune suppression to be strongly and independently associated with the risk of developing this post-transplant opportunistic infection. Albeit its severity, nocardiosis pathophysiology and predisposing factors remain largely unknown. Evidence available prior to the current study was very limited, with only small studies available (with ≤ 25 patients with invasive nocardiosis per study), and only one of them including a multivariable analysis looking at potential risk factors of nocardiosis after HCT. Most reported *Nocardia* infections among allogeneic HCT recipients occurred among patients with poor immune function, frequently suffering from GVHD.[5,15] Case control studies have provided contradictory results on the association between GVHD and nocardiosis.[10,11,16] In our analysis, GVHD was not identified as an independent risk factor for nocardiosis, but we identified use of tacrolimus, exposure to corticosteroids and lymphopenia as potential risk factors. Although tacrolimus use has been repeatedly associated with an increased risk of nocardiosis in the SOT recipient population,[8,17,18] data are more limited among HCT recipients. Although more than three quarters of *Nocardia* cases identified in some series received a tacrolimus-based regimen,[15,19] its use did not appear to be associated with nocardiosis by Yetmar and colleagues.[11] The same paradox applies to corticosteroids, despite their use in 75–100 % of *Nocardia* cases reported.[5,11,15,20–22] A single small case control analysis demonstrated the role of both tacrolimus and recent use of high dose corticosteroids in univariate analysis.[10] Cell-mediated immunity probably plays a major role in the protection against *Nocardia* infection[23]; in a small case series in the allogeneic HCT setting, all cases of disseminated nocardiosis occurred in patients with less than 100 $CD4^+$ T-cells/mm^3.[24] Although in vitro models have shown conflicting results concerning the role of neutrophils,[25,26] none of the *Nocardia* cases in our study were severely neutropenic,[6] and, in line with previous studies, we found lymphopenia but not neutropenia to be associated with the occurrence of nocardiosis after HCT.[5,20,22]

We also found CMV infection within previous 6 months to be independently associated with *Nocardia* infection. Conflicting results were reached in studies done in SOT recipients.[7–9,27] Very limited data are available among HCT recipients, where CMV infection within last 6 months has been found to be more frequent in patients with nocardiosis than in controls, in univariate analysis.[11] Whether our findings truly reflect a causal relationship or a bystander of a net state of immunosuppression remains to be determined. However, CMV was shown to interact with anti-infectious immunity and facilitate other opportunistic infections.[28]

Male sex was found in our study to independently associated with the risk of nocardiosis. This effect of biological sex is intriguing. Although a male predominance has been repeatedly observed in case series on nocardiosis,[16,19–21] case-control studies done in the HCT or SOT population did not identify male sex as a significant risk factor for nocardiosis.[8,9,11] However, it should be noted that male sex has been found to be associated with risk of invasive fungal disease, with behavioral and hormonal factors being suggested as potential explanations.[29]

Our study provides evidence that TMP-SMX prophylaxis, which is used in many centers to prevent post-transplant infections such as *Pneumocystis* and *Toxoplasma* infections, may also reduce the risk of post-transplant *Nocardia* infection. Previously published uncontrolled retrospective studies performed among allogeneic HCT also suggested that TMP-SMX could have a protective effect[19,30] and the potential protective effect of TMP-SMX is also supported by a case-control study done in 20 HCT recipients with nocardiosis and 80 controls.[11] Very recently, an individual patient data meta-analysis in which almost 800 SOT recipients were included found TMP-SMX to be associated with a significantly reduced risk of post-transplant nocardiosis.[31] Altogether, those results call for adherence to the ECIL *Pneumocystis* pneumonia prophylaxis guidelines and the preferential use of TMP-SMX for post-transplant prophylaxis,[32] underlining a possible collateral benefit against *Nocardia* infection in addition to other benefits already described, such as *Toxoplasma* prophylaxis.[33] Even if we found TMP-SMX prophylaxis to be significantly associated with a reduced risk of nocardiosis after adjustment for confounders, it is important to acknowledge that this effect is, at best, only partial. In fact, we and others have documented breakthrough *Nocardia* infections in patients receiving TMP-SMX, generally due to TMP-SMX-susceptible isolates.[31,34] As a consequence, and given the potential severity of *Nocardia* infection, we believe that a high level of suspicion is requested for nocardiosis in transplant recipients presenting with suggestive symptoms, independently of the use of TMP-SMX prophylaxis.

Our case-control study confirms that *Nocardia* infection is epidemiologically associated with an increased risk of death among HCT recipients, as compared with control HCT recipients. This finding is in agreement with previous studies in HCT and SOT.[11,16,35] Whether this increased mortality is directly related to nocardiosis or not is a subject of debate. In previous studies (including the parent study of the current one), death was often not attributed to nocardiosis by the treating clinician, and statistical analyses found death to be predicted by markers of poor general medical condition rather than severity of nocardiosis.

Our study has some limitations. First, due to its retrospective design, some data could not be retrieved. Specifically, information concerning environmental exposure were not available and could not be analyzed. Secondly, the exact dose of TMP-SMX used for

prophylaxis was missing for a significant number of cases and control subjects, making it difficult to assess the dose-response relationship of TMP-SMX in *Nocardia* prevention. Thirdly, some results of the multivariable analysis should be taken cautiously considering the relatively small sample size of the study and put in perspective with the already existing literature. It is comforting nevertheless that the protective effect of TMP-SMX in multivariable analysis (OR=0.23; 95 % CI = 0.07–0.81) was already manifest in bivariable analysis after adjusting for steroids intake (OR= 0.36; 95 % CI = 0.15–0.87) and comes as a confirmation of previous studies showing a similar protective effect.[11]

In summary, we identified ongoing use of tacrolimus, lymphopenia, recent receipt of systemic corticosteroids, CMV infection within the previous 6 months and male sex to be significantly and independently associated with the occurrence of nocardiosis in HCT recipients, while the use of TMP-SMX prophylaxis appeared protective. We also found HCT recipients with nocardiosis to have a significantly increased risk of death, as compared with matched patients who did not develop nocardiosis after HCT.

Funding

No funding was received for this study.

CRediT authorship contribution statement

Study conceptualization: AD, DA, DL, JC, JDG, JM, JS, LG, MM, NK, OL, RdlC; study supervision: AD, DA, DL, JC, and JDG; writing—original draft: DL, JC and JDG; writing—review and editing: all authors; statistical analysis: LT and AF; data extraction: TZ, XR, CR, AX, SP, YB, CB-G, NKh, ALB, JVP, AH, NK, SDL, DR-W, MA, NB, IWB, KC, MC, AG, AV, JL, SM, AN, RR, AT, MS, LW, GT, NK, JM.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106162.

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